ON A SINGULAR OVARIAN CHANGE PRODUCED BY METHYLANDROSTENEDIOL (MAD)

BY

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METHYLANDROSTENEDIOL (MAD) possesses rather unusual pharmacological actions. In the adrenal cortex of the rat it causes a deposition of numerous hyalin, PAS-positive droplets. This change is accompanied by hypertension, nephrosclerosis and cardiac lesions, similar to those normally elicited by overdosage with desoxycorticosterone acetate (DCA). However, MAD —unlike DCA—fails to produce any of these cardiovascular and renal lesions in the adrenalectomized animal maintained on NaCl or on small doses of corticoids. Hence, it was assumed that MAD acts by producing some derailment in the steroid-hormone metabolism of the adrenal cortex (Salgado and Selye, 1954a; 1954b; 1954c). It has also been shown that MAD interferes with the normal corticotrophic effect of exogenous A.C.T.H. (Selye and Bois, 1954).

In the course of various experiments it had been noticed, incidentally, that the ovaries of rats receiving large doses of MAD become intensely hyperaemic while the corpora lutea assume a singular and quite distinctive whitish colour. Even the most superficial histological examination of these ovaries revealed changes in their microscopical structure, such as we had never observed before, under any other experimental conditions. It has therefore been decided to undertake a systematic study of the ovarian lesions produced by MAD in normal rats, as well as in adrenalectomized animals maintained by various corticoids.

METHOD

Forty female Sprague-Dawley rats were subdivided into four groups of 10 animals each as outlined in our Table. The mean initial bodyweight was 160 g., in all four groups, with a maximum range of 10 g. above or below the mean.

MAD (17α-methyl-Δ^5-androstene-3β, 17β-diol) microcrystals were administered subcutaneously at the daily dose-level of 5 mg. in 0·2 ml. of water, "Tween 80" being used as a suspending agent.

*Cortisol** (400 μg/day) and *desoxycorticosterone* (100 μg/day) were both given in the form of microcrystals of their acetates, subcutaneously in 0·2 ml. of water, using the same suspending agent.

Adrenalectomies were performed through the dorsal route, under ether anaesthesia, on the same day on which treatment with the various steroids began. Ten days later the animals were killed with chloroform and their ovaries fixed in "Susa" solution for subsequent weighing and histological study.

RESULTS

The general plan of the experimental arrangement, as well as the ovarian weights (with their standard errors), are summarized in Table I.

The ovarian weight was only slightly decreased by MAD; however, even mere macroscopic inspection showed that the ovaries were very hyperaemic and the corpora lutea had a characteristic ivory-white colour.

Upon histological examination, it was found that, in all individuals of the three MAD-treated groups, the same type of ovarian lesion had

* In agreement with the suggestion made by Shoppee (1953), the term cortisol is used here in preference to "hydrocortisone", since it eliminates confusion with 4,5-dihydrocortisone and obviates the possible implication that "hydrocortisone" is to be regarded primarily as a mere derivative of cortisone.

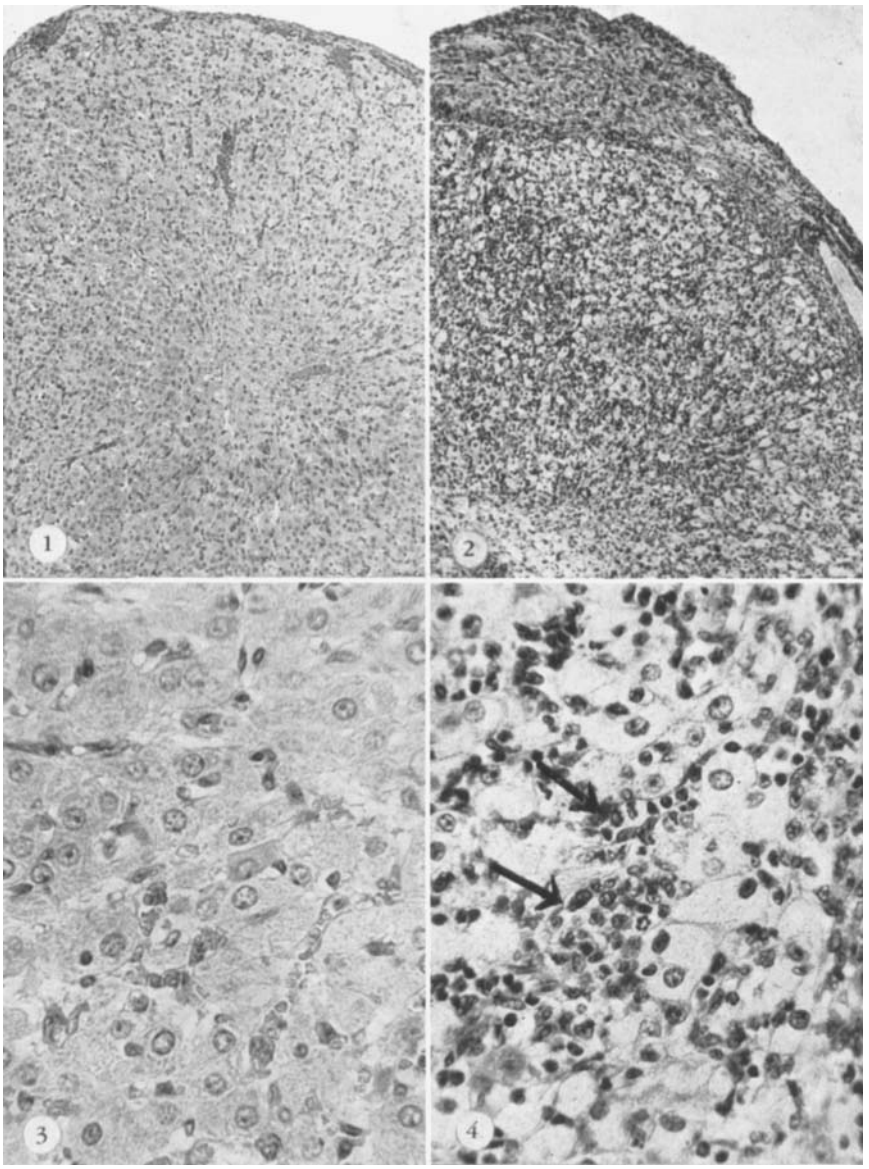

FIG. 1

General aspect of a normal corpus luteum from a rat of Group 1. ×75.

FIG. 2

General aspect of a corpus luteum in an MAD-treated normal rat of Group 2. Note the intense infiltration with small dark cells between which some enlarged very clear "foam-cells" are especially conspicuous. ×75.

FIG. 3

Region from the corpus luteum shown in Fig. 1 to illustrate normal aspect of individual corpus luteum cells at higher magnification. ×337.

FIG. 4

Region from the corpus luteum shown in Fig. 2. Note large "foam-cells" with comparatively dense nuclei and the infiltration of dark small cells between them. Note also numerous lymphoid and polymorphonuclear cells, both in the capillaries (upper arrow) and in the stroma between the foam-cells (lower arrow). ×337.

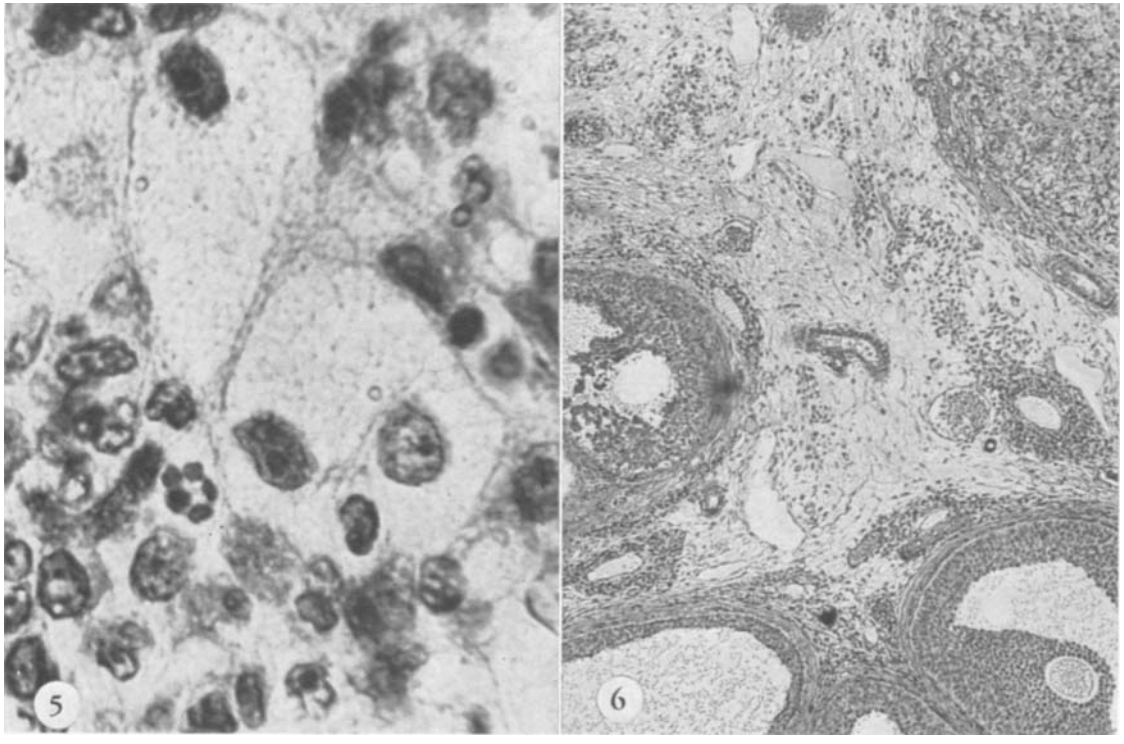

FIG. 5

Detail from the central field of Fig. 4. "Foam-cells" with their dense eccentric nuclei as well as the lymphoid and polymorphonuclear cells are visible. $\times 1000$.

FIG. 6

Region from the medullary portion of the ovary in an MAD-treated normal rat of Group 2. Note the hyperaemia and oedema of the stroma, the great atrophy of the interstitial cells (distinguishable only as small dark dots within the oedema of this connective tissue) and the typical infiltration of a corpus luteum (part of which shows in the upper-right corner of the field) are all clearly visible. The granulosa of the follicles in the lower part of the field is unchanged. $\times 100$.

TABLE I

Effect of MAD upon Ovarian Weight in Intact and in Adrenalectomized (Corticoid-treated) Rats

Group	Treatment	Ovaries
I	Normal, Untreated	47·4±3·4
II	Normal, MAD	38·2±2·2
III	Adrenalectomized, Cortisol, MAD	39·2±4·9
IV	Adrenalectomized, Cortisol, DCA, MAD	37·5±2·6

developed. These appeared to be least severe in the intact (Group II) and most pronounced in the adrenalectomized animals receiving both cortisol and DCA (Group IV), but individual variation was quite marked, within any one group. Since, furthermore, these morphological changes do not lend themselves to statistical evaluation, this apparent difference in the severity of the lesions may be coincidental.

Histologically, the typical lesion is primarily characterized by an extremely pronounced dilatation of the ovarian veins. This is manifest in the medullary portion of the gland, where the large sinuses are situated, but also in the stroma, the theca nests and the corpora lutea, which contain smaller veins. Recently developed cystic corpora lutea (which have not yet accumulated many lipid droplets) are much less markedly affected by this venous stasis than the fully developed, or involuting, older corpora lutea. Occasionally, extremely dilated sinuses are also seen underneath the ovarian capsule, on the surface of large corpora lutea. Indeed, these may become wide enough to be clearly visible to the naked eye.

The theca-cell nests ("interstitial gland") within the ovarian stroma also show another characteristic change, namely, a pronounced involution of the epithelioid cells, with a nuclear rearrangement, in such a manner that the clear spaces between the basophilic chromatin assume the appearance of the spokes of a wheel. Similar cells have been observed in the ovaries of hypophysectomized rats and were given the name "wheel cells". They are somewhat reminiscent of plasma cells and usually occur as a result of LH deficiency (Selye, Collip and Thomson, 1933). In some animals the stroma between the theca-cell nests became extremely oedematous.

The most typical changes occur, however, in the parenchymatous elements of the corpora lutea themselves. Here, the entire tissue appears to be transformed into a structure which is singularly reminiscent of the xanthomatoses, for instance, of Niemann-Pick's disease. Some of the corpus luteum elements retain many of their original characteristics, but become somewhat enlarged and laden with light vacuoles, so that they assume the appearance of "foam-cells". On frozen sections stained with Sudan III, these vacuoles stain a bright red; they presumably contain lipid materials. Between the foam-cells, there is an infiltration of much smaller, rather irregular elements, containing little or no lipid. At certain places, they resemble lymphocytes, monocytes or histiocytes, but occasionally there are typical polynuclear leucocytes among them. This picture of large lipid-storing cells, between strands of small lymphoid or haemopoietic-tissue-like elements, also bears a striking resemblance with the changes in the adrenal cortex which we had observed in tumour-bearing rats and in animals given chronic treatment with large doses of STH (Selye, 1950). Sections stained with the McManus technique (McManus, 1948) revealed no PAS-tingible granules, comparable to those seen in the adrenals of MAD-treated rats (cf. Figs. 1–6).

The uteri and the preputial glands of all the MAD-treated animals were markedly enlarged and there was also some mammary-gland proliferation. However, from the appearance of these organs we cannot draw any definite conclusions as to possible hormonal derangements resulting from this action of MAD upon the ovary.

In the present communication we merely wanted to point out that MAD, which had been shown to cause a marked derangement in the histological structure and steroid metabolism of the adrenal, also produces a rather characteristic morphological change in the ovaries. We had not seen any comparable lesions previously in rats treated with a variety of related steroids, such as testosterone, androsterone, methyltestosterone, etc. Chronic treatment with testosterone propionate can cause ovarian atrophy (Selye, 1940a; 1940b; 1941a; 1941b), as well as the formation of large corpora lutea and follicle cysts (Selye

and Friedman, 1940), but these changes are qualitatively different from those produced by MAD.

Summary

In albino rats, overdosage with methyl-androstenediol (MAD) produces a singular and rather characteristic ovarian change not previously observed to occur after treatment with pharmacologically and chemically related steroids. There is marked venous hyperaemia in the ovarian stroma, in the theca nests and in the corpora lutea. The microscopical appearance of the corpora lutea is also changed by the development of numerous large lipid-storing "foam-cells", between which there is a dense infiltration with darker elements, resembling monocytes, lymphocytes and sometimes polymorphonuclear leucocytes.

Previous work had shown that MAD produces quite characteristic changes in the adrenal cortex, with the accumulation of PAS-positive droplets. These are accompanied by renal and cardiovascular lesions, which are prevented by adrenalectomy. On the other hand, the above-mentioned ovarian changes are not influenced by removal of the adrenals, even in rats kept in good condition by maintenance doses of corticoids.

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